Isolation and Characterization of Nephropathogenic Strain of Infectious Bronchitis Virus in EGYPT

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Abstract: Three strains of infectious bronchitis virus with sever Nephropathological characteristics were isolated from Mansoura, Gharbia and Giza governorates early 2010. Egg propagation revealed the ability of the isolates to make mortalities of the SPF embryos by the 3rd passage after 72 h post inoculation. The embryos showed stunted growth with sever renal damage and deposition of ureates within the ureters and urinary bladder. PCR amplification of the 976bp of S1 gene gave appositive amplicon with 10^{-2} – 10^{-5} diluted infected allanotic fluid. phylogenetic analysis revealed that these strains are related to the IS/1494/06 nephropathic IBV strain[journal of American Science 2010; 6(9):669-675]. (ISSN: 1545-1003).

Keywords: infectious bronchitis; Nephropathological; Egg; nephropathic IBV strain.

1. Introduction:

Infectious bronchitis-the prototype of the Coronaviridae- is an acute and highly contagious respiratory disease of chickens. The disease is characterized by respiratory signs including gasping, coughing, sneezing, tracheal rales, and nasal discharge. In young chickens, severe respiratory distress may occur; while in layers, respiratory distress, decrease in egg production, and loss of internal and shell quality of eggs are reported.. Several strains of infectious bronchitis virus have a strong affinity for the kidney (nephropathogenic strains) and may be associated with high mortality(*Gorgyo et al., 1984)*. These strains may cause severe renal damage. This affinity for kidney tissue may have resulted from mutation as a result of selection pressure following widespread use of the modified live infectious bronchitis vaccines. That is, after prolonged use of live infectious bronchitis vaccines, which provided protection against infectious bronchitis virus infection in respiratory tissues, new tissues where little protection was present were infected as a result of viral mutation.

Infectious bronchitis is belong to group 3 coronavirus (*Cavanagh, 2003*). It is an enveloped, nonsegmented, positive sense single stranded RNA virus. IBV genome consists of about 27 kb and codes for three structural proteins: the spike (S) glycoprotein, the membrane (M) glycoprotein, and the nucleocapsid (N) phosphoprotein. The S glycoprotein is composed of two glycopolypeptides: S1 and S2 (*Cavanagh, 1983*). Neutralizing and serotype-specific antibodies are directed against the S1 glycoprotein, and the greatest divergence in the amino acid sequence is concentrated between residues 53 and 148 of S1 (*Wang et al., 1997*). The number of IB serotypes appears to have increased in recent years .Molecular studies have shown that a new IB serotype can emerge as a result of only a very few amino acid changes in the S1 part of the spike genome of the virus (*Cavanagh 1995*).

Infectious bronchitis virus has the ability to mutate or change its genetic makeup readily. As a result, numerous serotypes have been identified and have complicated efforts at control through vaccination. Three common serotypes in North America are the Massachusetts, Connecticut, and Arkansas 99 infectious bronchitis viruses. In Europe various "Holland variants," usually designated using numbers (D-274, D-212), are recognized (*Bayry et al., 2005)*.

Controlling IB infection is a problem due to wide variations in the serotypes and virulence of strains that have developed from time to time, a highly contagious nature, and the evolution of specific tissue tropism and recombinants due to simultaneous infection of multiple virus types and use of live vaccines (*Bayry et al., 2005*) . Outbreaks can occur in vaccinated flocks due to the lack of cross-protection against antigenically unrelated serotypes and variant strains of the virus (*Gelb et al., 1991; Capua et al., 1994; Jia et al., 1995*). Early study by *Jungherr et al. (1956)* showed that there are sufficient immunological differences among the strains; so that cross-protection would not occur. Other investigations showed that vaccines can elicit protection against some field challenges (*Davelaar et al., 1984; Parson et al., 1992; Afanador, 1994; Wang et al., 1997*). other investigations showed that this occurs in function of the genetic variability of originating variant strains *(Wang et al. 1997)*. Many authors propose that the inefficiency of the

vaccination programs is due to the large diversity of antigenically different strains; because IBV presents the phenomenon of genetic recombination or the virus can suffer a mutation, generating new strains (*Kouwenhoven and Davelaar, 1989*). Variant strains of IBV have been recovered from vaccinated flocks despite the use of combinations of several strains of live and attenuated IBV vaccines (*Gelb et al., 1991*). In the current study, isolation and phylogenetic analysis of Nephropathological strain isolated from 3 farms in 3 different governorate in Egypt have been done.

2. Materials and Methods:

Samples:

Kidneys were collected aseptically from commercial broiler and layer poultry flocks of 2-18 weeks of age in Mansoura , Gharbia and Giza governorates early 2010 . The broiler flocks demonstrating a respiratory disorders, anorexia, loss of bodyweight, possibly associated with increased mortality, and the layer flocks showing aberrant egg production. Nephropathological lesions were the predominant lesions found in these flocks.

Enzyme Linked Immunosorbent Assay.

A commercial ELISA kit from Kirkegaard & Perry Laboratories, Inc. (KPL), Gaithersburg, Maryland, USA, was used to determine IBV antibodies in sera of chickens, according to the manufacturer's instruction.

Virus isolation using Embryonated SPF eggs.

(Momayez et al., 2002)

The samples were homogenized to give approximately 10% (w/v) suspension in PBS pH 7.2 containing 100IU/ml penicillin, 100µg/ml streptomycin, and 30 IU amphotericin B/ml. The homogenized samples were centrifuged at 1000g for 15min at 4°C and then filtered through a 0.45µm filter membrane The supernates was inoculated at 0.2ml via the chorioallantoic cavity of groups of ten 9-11 day-old SPF eggs.

Inoculated eggs were checked twice a day. Those that died within 24h after inoculation were discarded. Mortality between 2 and 7 days post inoculation (PI) were considered to be virus specific. The chorioallantoic fluid was harvested aseptically from embryos that died between 48 and 72h PI, if the fluid showed no Hemaglutination (HA) activity.

Dead embryos were examined for the presence of embryo stunting, curling, urate in the mesonephros, or focal necrosis in the liver. On day 3 PI, five live embryos were also removed from the incubator and were placed at 4°C for 24h and the chorioallantoic fluid of the embryos was collected for the next passage.

Polymerase chain reaction:

The allanotic fluids collected at 4 days post-inoculation was serially diluted and used in reverse transcription-polymerase chain reaction (RT-PCR). Briefly, total RNA extracted by triazol method (according to the manufacture instruction) from the infected allanotic fluids was reverse transcribed by H minus first strand cDNA synthesis kit (Fermentas, USA). The cDNA was used with 'S1' gene specific primers:

Sense 5'- TGCCTCCGAGTGTTCTGTGGGT-3'

antisence 5'- ACACCCTCCCTGTAGGGGTCCA-3'

for PCR amplification of product (976 bp) with the following cycling conditions: initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 45 sec; 60°C for 1 min; 72°C for 2 min, followed by a final extension of 72°C for 7 min.the Allantoic fluid was tested also for the presence of other viral contaminants using specific primers for NDV, AI and IBD

Phylogenetic analysis:

It was done in OIE , FAO and National References Laboratory For Newcastle disease and Avian influenza, Viale Dell' Universita, 10 – 35020 LEGNARO (PD)

3. Results:

Egg passage

After the third passage in specific-pathogen-free embryonated eggs, lesions were observed in the specific-pathogen-free embryos in the form of mortality of embryos, stunting, curling (Fig 1), and uric acid deposition in the kidneys and ureter (Fig 2). The allantoic fluid of inoculated eggs were found to be negative for Newcastle disease virus and avian influenza virus by Hemaglutination assay.

Polymerase chain reaction:

Amplification of the S1 gene from the infected allanotic fluid resulted in a 967 bp amplicon (does not appear with the uninfected Allantoic fluid). The positive results was seen with 10^{-2} to 10^{-5} dilutions of Allantoic fluid . further dilutions could not yield any PCR products.

No amplifications were seen when using primers for NDV, AI and IBD viruses.

Phylogenetic analysis:

The strains isolated from the three governorates shows homology to the IS/1494/06 strain (blast search)

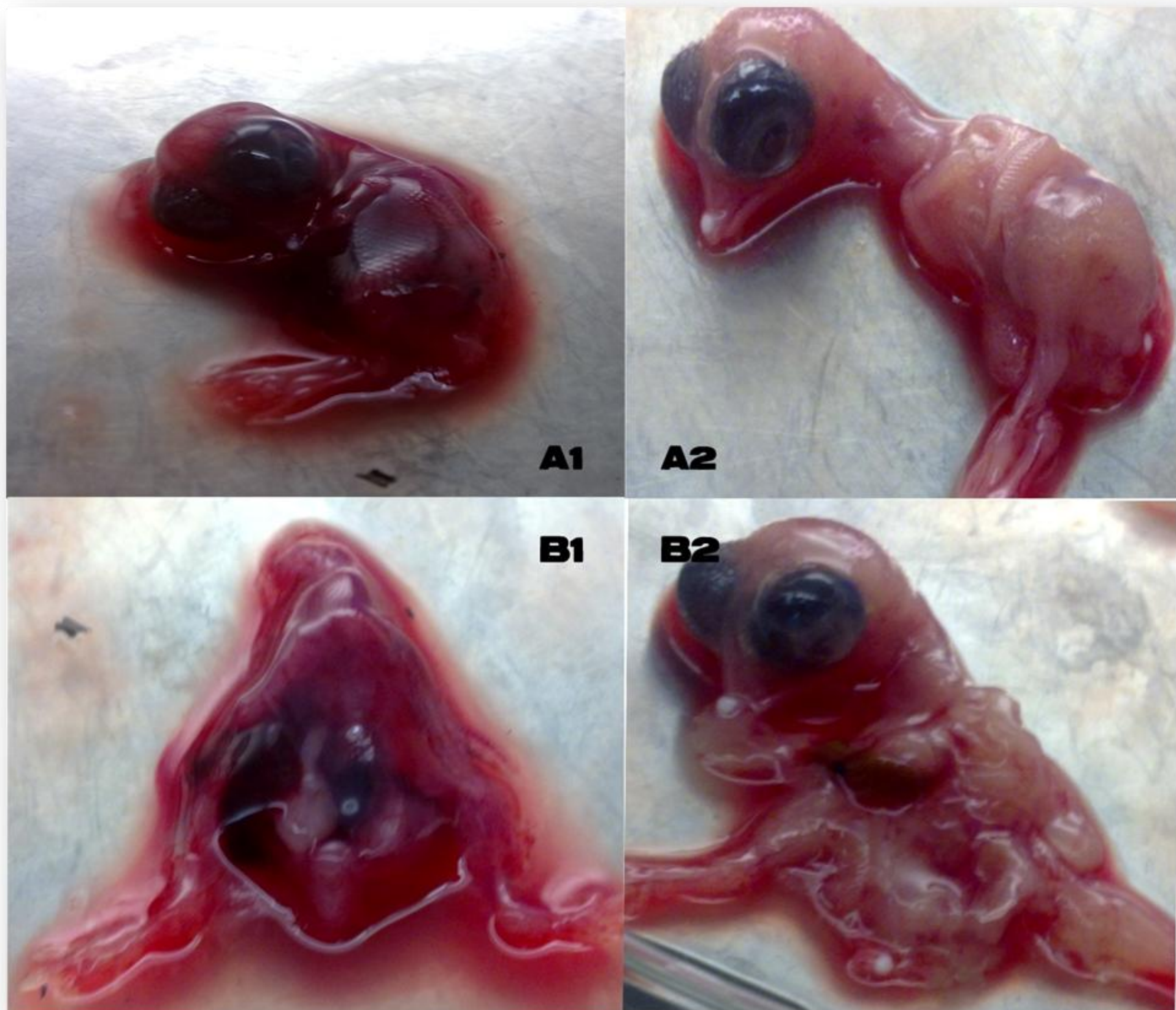

Fig (1) showing the stunted growth and the curled appearance (A1) and the severely inflamed kidneys (B1) of the embryo due to IB inoculation of 9 days old SPF chick embryo compared with the non inoculated one (A2 and B2).

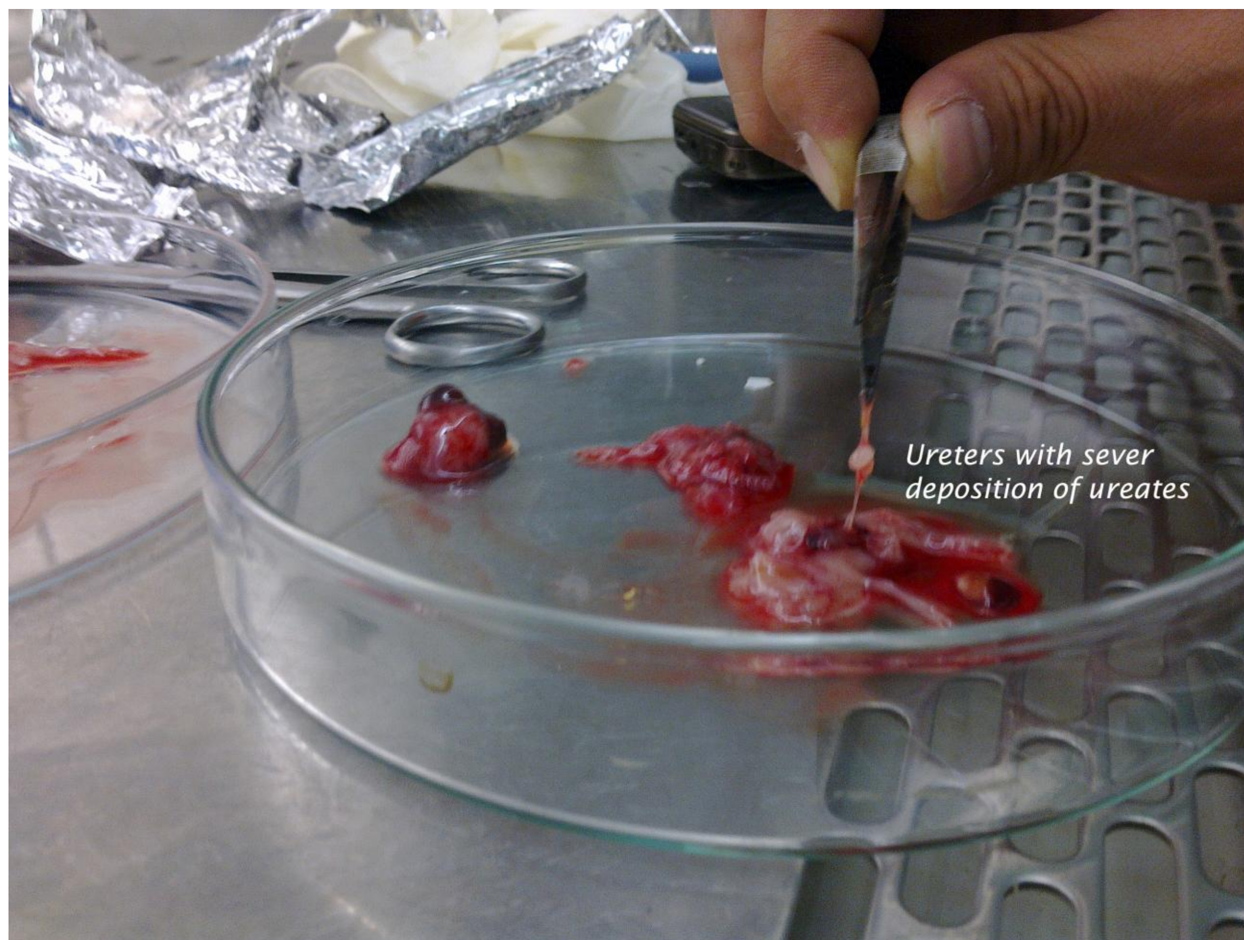

Fig (2) showing the ureters with deposition of urates in embryo due to IB inoculation of 9 days old SPF chick embryo .

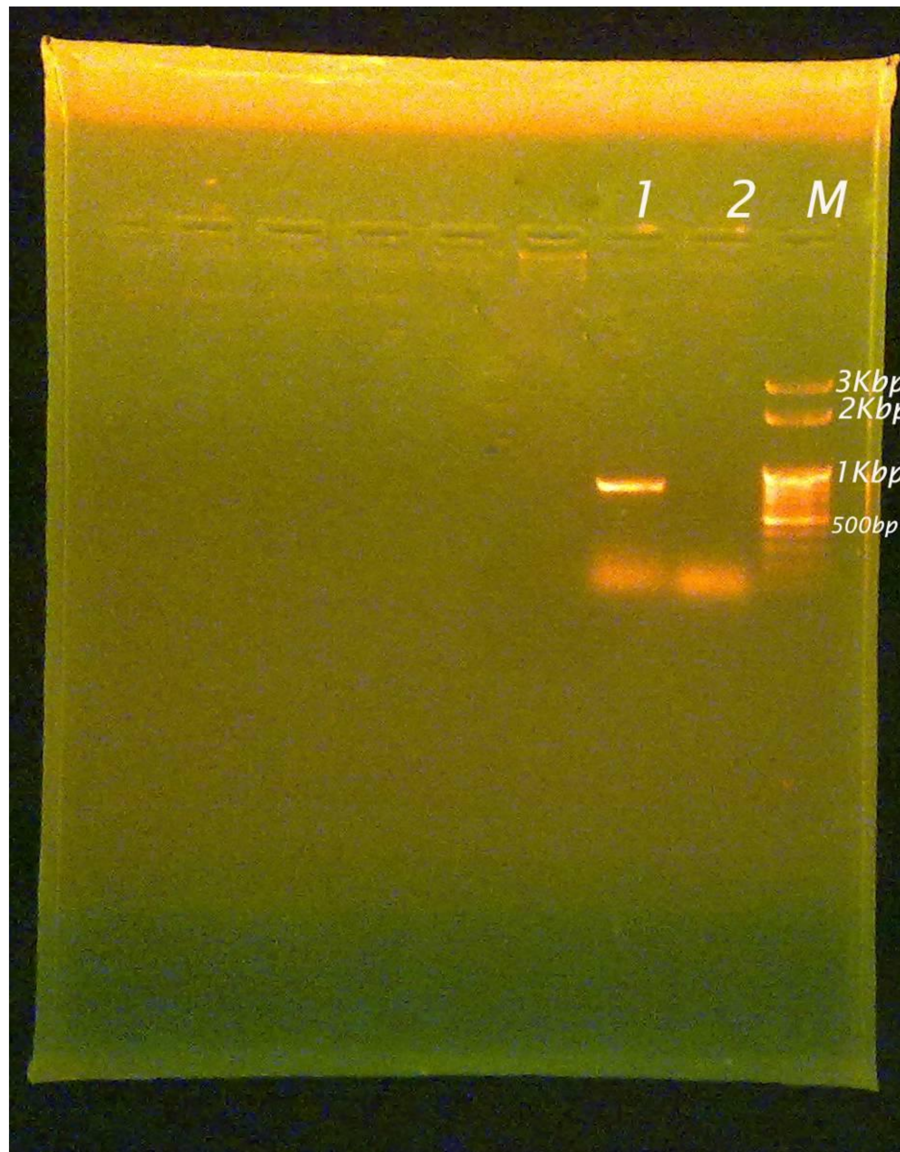

Fig (3) amplification of S1 segment of the IBV showed a product size of 976 bp with the IBV infected chorioallantoic fluid(lane 1) while no infected alantoic fluid showed only the primer dimmer (lane 2) . (M) is a 100bp DNA marker.

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4. Discussion:

The most prevalent viral respiratory pathogen in chickens are Newcastle disease, Avian influenza and Infectious bronchitis viruses. The incidence and isolation of IBV has been rare, probably due to the ubiquitous presence of ND viruses that masked IBV infections. Now extensive vaccination with several types of ND vaccines is routinely being used, bringing the disease largely under control. In this context, IB has started causing great economic losses. Massive vaccination with the MS(H120) strain lead to the apparent control of the IB infection in Egypt but it may lead to emergence of IBV strains with different tissue tropism

IBV exists as many serotypes that can be identified by virus neutralization (VN) test (*Ambali and Jones, 1990; Calnek 1997*). It is known that theS1 subunit was involved with infectivity and

haemagglutinin activity and carries serotype-specific sequences (*Cavanagh and Davis, 1986; Cavanagh 1995*) and antigenic epitopes inducing virus neutralizing antibody. The different serotypes, subtypes or variants of IBV was thought to be generated by nucleotide point mutations, insertions, deletions or RNA recombinations of S1 genes (*Cavanagh 1983; Cavanagh and Davis, 1986; Jia et al., 1995*), which were responsible for outbreaks of IB in the vaccinated chicken flocks. In addition to serotype changes, the genetic variation may result in changes of the tissue tropism and pathogenicity of the virus, which lead to the generation of new IBV pathotypes.

The isolate recovered from kidney of the IB vaccinated flock was shown to produce dwarfing in the infected embryos and showed similar characteristics of coronavirus IBV such as size in diameter, morphology, and haemagglutinatiing properties. The clinical manifestations, gross and microscopic lesions of the infected chickens were the same as those infected with nephropathogenic IBVs (*Calnek, 1997; Zhou and He 2000*), and the strains was re-isolated from the damaged kidneys of the infected chickens. RT-PCR products of S1 gene of the isolate were obtained using specific primers of IBV.

Inoculation in the SPF eggs resulted in stunted growth of the embryos – a characteristic sign for the IBV – plus sever nephropathic changes that may resulted due to change in the tissue tropism of the virus with replacement of the predilection site - trachea and respiratory mucosa – to another tissue like urinary epithelia.

Phylogenetic analysis revealed that all the isolates sequenced had homology to the IS/1494/06 nephropathic strain (blast search) but were different from that of the H120 vaccine virus. Thus it appears that the vaccine viruses are acquiring point mutations and insertions during its spread in the field. Although speculative, it is possible that in due course, the accumulation of these mutations may lead to viruses that may escape protection by existing vaccines.

In conclusion, the isolated virus from chickens suffers from severe renal lesions was found to be nephropathic variant of Infectious bronchitis virus related to the IS/1494/06 nephropathic strain.

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